RNA-interference (RNAi) and control of the glassy-winged sharpshooter (*Homalodisca vitripennis*) and other leafhopper vectors of Pierce's Disease

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Specific objectives

- To identify and develop RNAi-inducers capable of killing or reducing the survival and/or fecundity of *Homalodisca vitripennis.*
- To generate transgenic plants capable of expressing and delivering *Homalodisca vitripennis* deleterious RNAi molecules within their xylem.
- To evaluate transgenic plants for their ability to generate RNAs capable of inducing RNAi vs. *Homalodisca vitripennis*.

Why did we choose to focus our project on the control of the PD vectors, instead of on another aspects of the PD disease cycle?

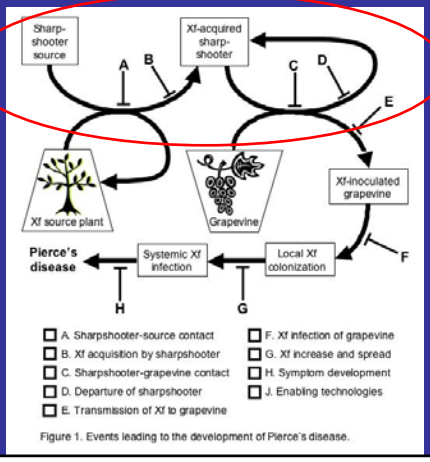


Figure 1. Events leading to the development of Pierce's disease.

Challenges of working with GWSS

- GWSS are quarantined in northern California.
- GWSS are difficult to rear in artificial environments.
- GWSS tend to go in ovipositional diapause in winter months.
- GWSS so far do not like artificial diets.

- BUT, so far so good, we are still getting nymphs, plus we are now also rearing *Draeculacephala minerva* **(non BSL 3P)**

UC Davis Biosafety Level 3P Contained Research Facility

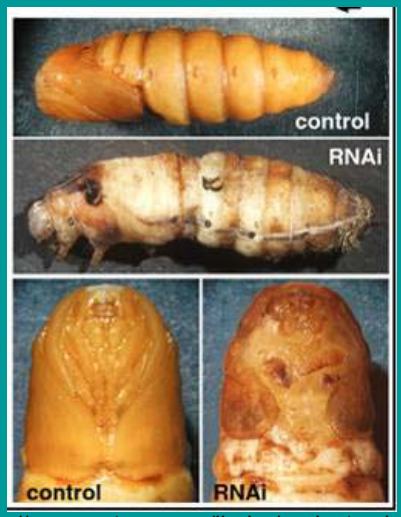
Why RNAi?

- Applications of RNAi technology-based strategies for insect and disease management are becoming more widespread.

Three papers in *Nature/Biotechnology* last month, November 2007:

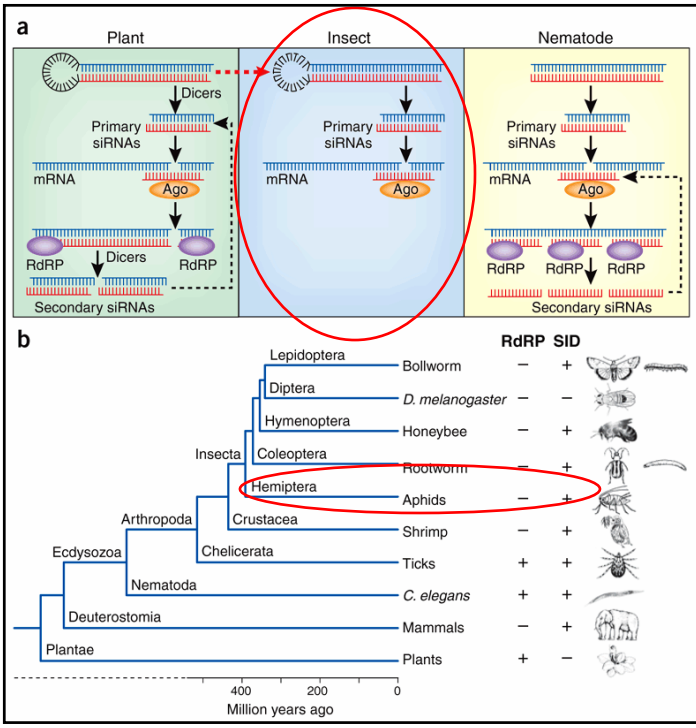
1) **RNAi for insect-proof plants - pp1231 – 1232,** Karl H J Gordon & Peter M Waterhouse
2) **Silencing a cotton bollworm P450 monooxygenase gene by plant mediated RNAi impairs larval tolerance of gossypol - pp1307 – 1313,** Ying-Bo Mao, et al.
3) **Control of coleopteran insect pests through RNA interference - pp1322 – 1326,** James A Baum, et al.

The example below shows RNAi effects in the silkworm, the target was BR-C, involved in silkworm metamorphosis.



http://www.entu.cas.cz/jindra/projects.php

We will utilize RNAi strategies against *H . vitripennis.* We will test dsRNAs, and miRNAs for targeting specific *H . vitripennis* genes.

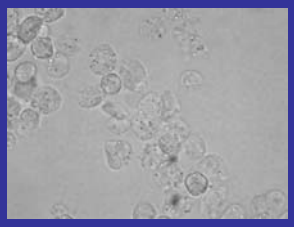


Is there evidence for a functional gene silencing, RNAi pathway in GWSS?

RNAi for insect-proof plants. Nat Biotechnol. 2007 Nov;25(11):1231-2.

Systems used in our studies

- GWSS cells



GWSS cells were provided by Drs. G. Kamita and B. Hammock UCD

- GWSS insects

GWSS insects were originally provided by Dr. R. Almeida UCB

Our original approach.

We already have made significant progress on several steps and anticipate more accomplishments before the end of the year.

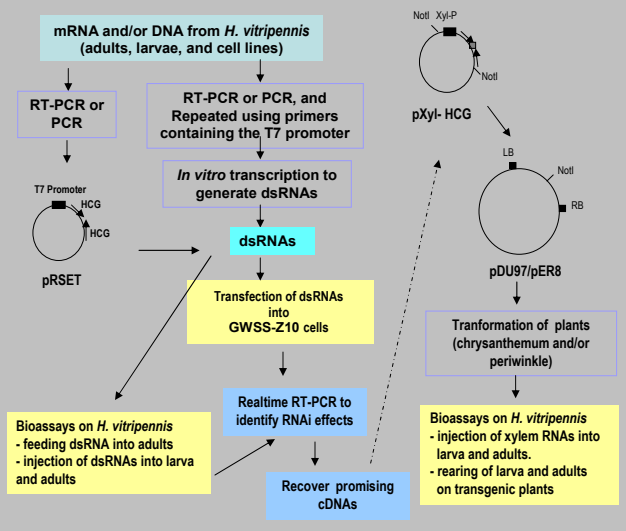


Figure 2. Synthesis of RNAi target genes, production of dsRNAs, and bioassyas. pRSET is an *E. coli* expression vector; pXyl-HCG is a modified version of pRNA69, containing the xylem specific promoter (EgCAD2) and *H. vitripennis* gene sequences (HCG). pDU97 and pER8 are binary vectors for plant transformation and contain NPTII and HPTII as a selectable markers for kanamycin resistance and hygromycin resistance, respectively.

We can take advantage of the abundant and rapidly accumulating sequence data for many insects such as *Drosophila* and even GWSS.

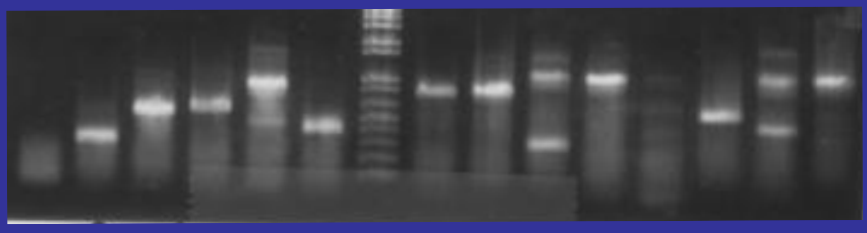
We have initially targeted two genes as will be shown below (actin and Sar1).

Interfering with either of these results in responses we hope to assess by RNA levels and phenotypic response.

We will initially test delivery by injecting interfering RNAs and feeding them *in vitro* (via the xylem) to GWSS.

We will perform these experiments in the lab (GWSS cells) and in the UC Davis, Biosafety 3P Contained Research Facility.

Genes screened in GWSS as candidate gene silencing targets.



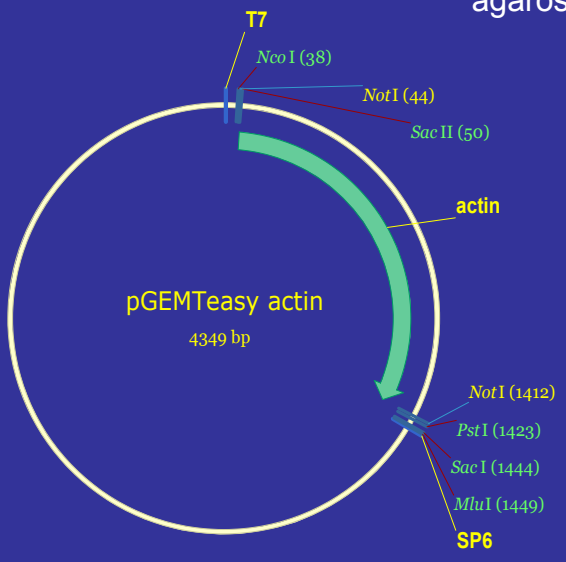
One-Tube RT-PCR products obtained using specific primers designed based on GenBank available EST sequences

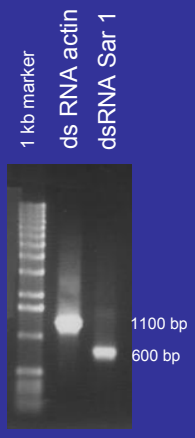
First genes chosen for their cellular activities to trigger gene silencing.

- 4 genes for **Actin**, a contractile protein found in muscle cells. Together with myosin, actin provides the mechanism for muscle contraction.
- **Sar1** gene is involved in transport from the endoplasmic reticulum to the Golgi apparatus. It belongs to the small GTPase superfamily. SAR1 family.

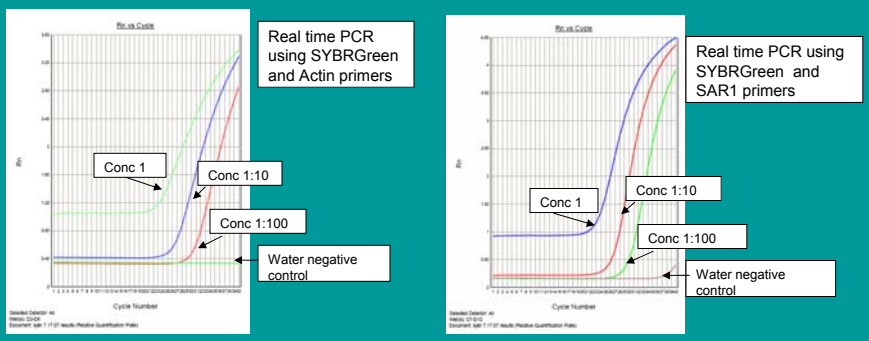
dsRNA constructs

- *In vitro* transcripts were annealed and examined by agarose gel electrophoresis



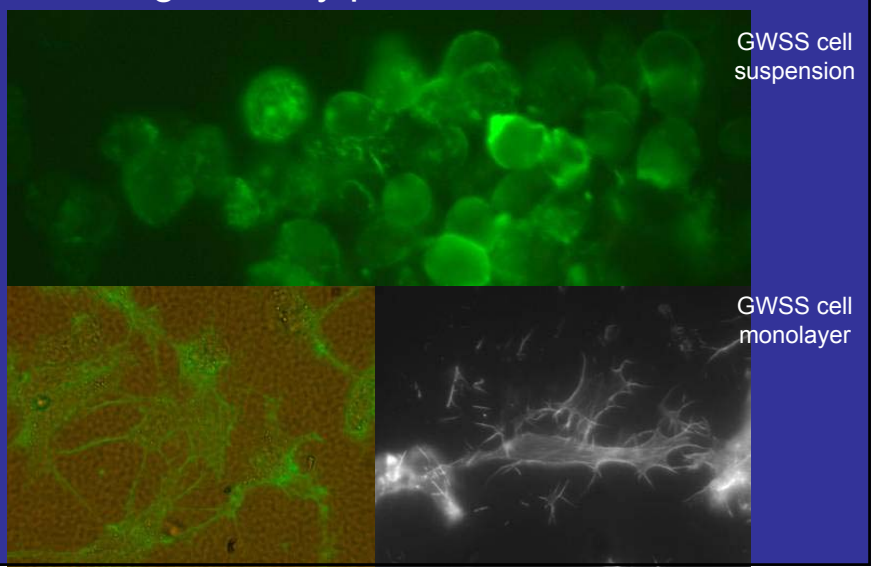


Detection of Actin and Sar1 mRNA from GWSS ZW-20 cells can be done by Northern blot, siRNA detection and RealTime RT-PCR.

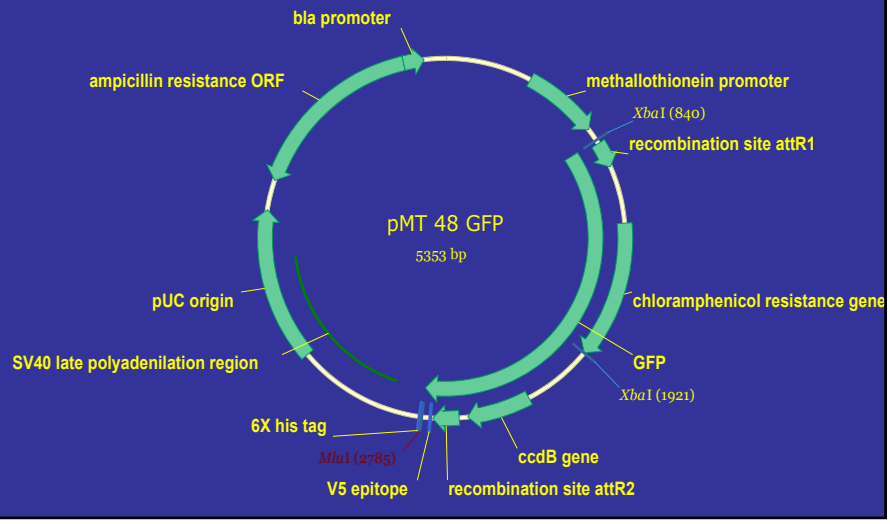


Real Time PCR using SYBRGreen. On the left: Actin primers. On the right: Sar1 primers

Actin can be stained using Alexa-Fluor phalloidin (Molecular probe, Invitrogen), a high-affinity probe for F-actin

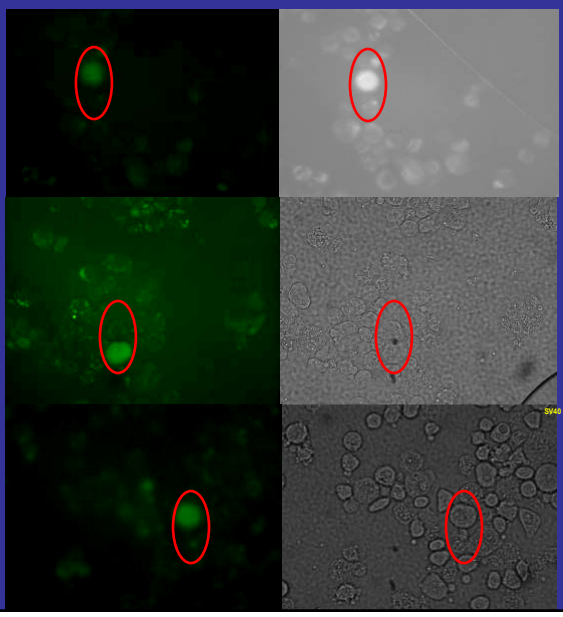


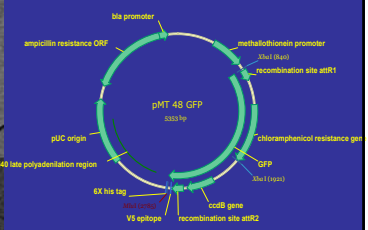
We can trasnfect GWSS cells using a GFP inducible plasmid.



- Transfection efficiency was ~5% in initial experiments

We are working to make it better.

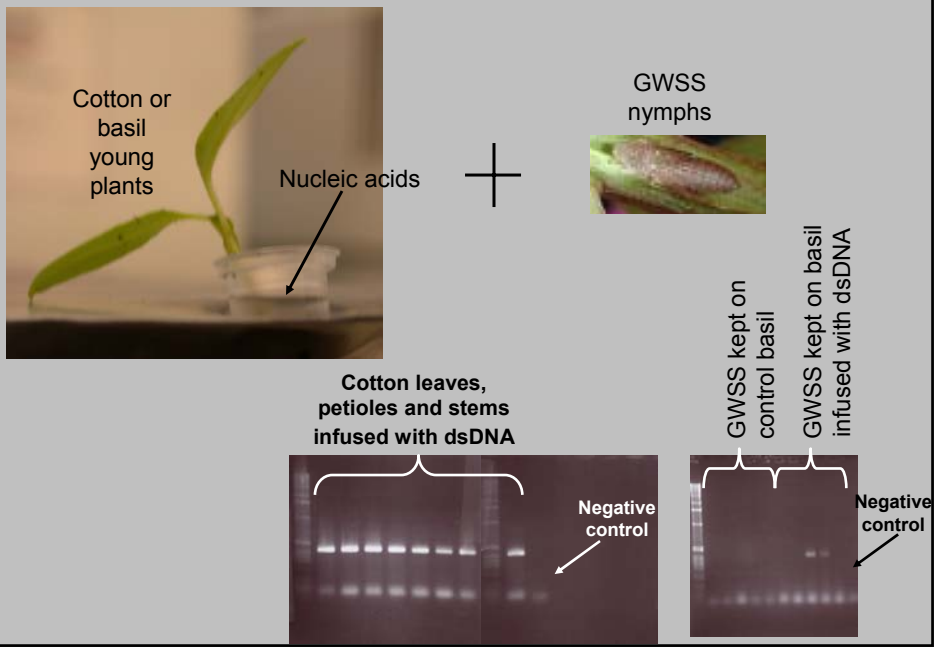




In vivo experiments: dsRNA delivery systems used in UCD-reared GWSS nymphs

- Injection
- Feeding

dsDNA plant infusion.



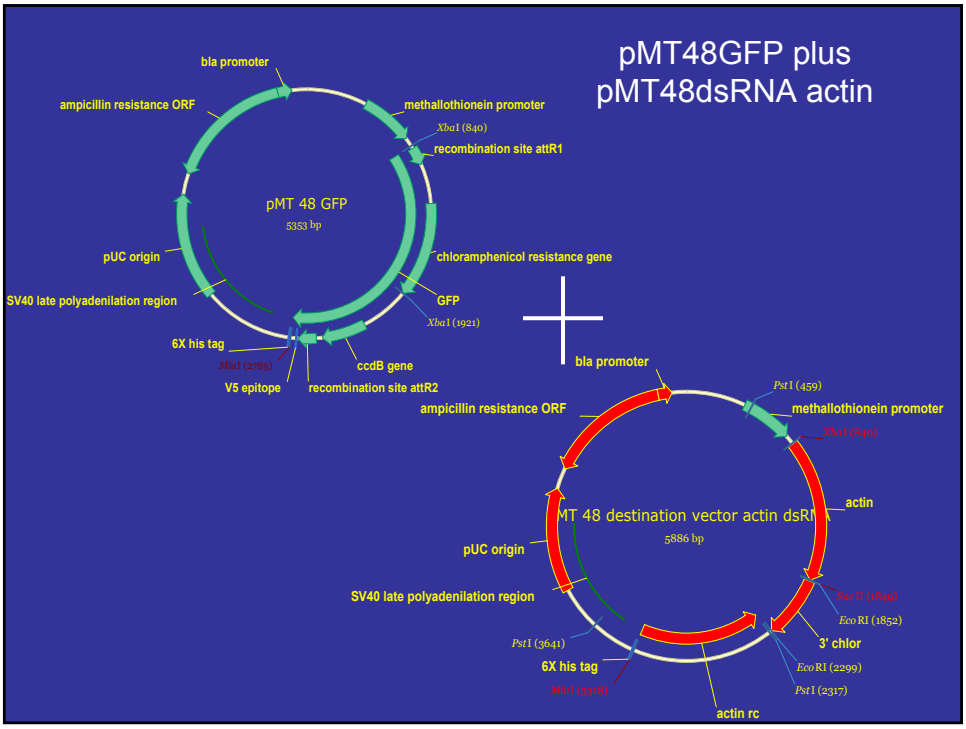
Injection of 1µg/µl of Actin dsRNA into GWSS insects

11/20/2007	control injection buffer	dsRNA actin injection
insects observed on day 1 11/21	5	8
day 2 11/22	4	8
day 3 11/23	3	5
day 4 11/24	3	4
day 5 11/25	3	3
day 6 11/26	3	2
day 7 11/27	3	0

We are developing assays for RNAi effects, and improving our injection technique.

Ongoing experiments:

- Repeating dsRNA injections on GWSS, try new dsRNA constructs, extend experiments also using the grass sharpshooter (*Draeculacephala minerva*) PD vector.
- Starting dsRNA infusion on feeding plants.
- Testing for presence of induced siRNA in dsRNA injected insects.
- Constructing a hairpin loop actin plasmid to create a stable cell line and to use in co-transfection experiments



…..Then perform cell staining with phalloidin (red fluorescent dye)

- Cell transfected will be green (GFP) and their actin will be stained red.

Acknowledgments :

- Ho Chuen Tsui
- CRF staff
- Drs. Rodrigo Almeida, Elaine Backus, Sandy Purcell, George Kamita, Bruce Hammock